In-depth genomic characterisation of SARS-CoV-2 variants from Egypt exhibited heterogeneous lineages

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Abstract

Background: Genetic variation of SARS-CoV-2 remains a public health challenge worldwide because of it influences the pathogenicity and transmissibility of the virus.

Aims: To determine the genomic mutations of SARS-CoV-2 isolated in Egypt.

Methods: This was an *in silico* cross-sectional study of 200 SARS-CoV-2 variants of concern, which were retrieved from the National Centre for Biotechnology Information on 11 January 2021 and aligned with the original Wuhan strain (NC_045512). Nucleotide basic local alignment search tool (BLASTN) was used to identify the nucleotide variations. Corresponding proteins were identified using protein basic local alignment search tool (BLASTP). A phylogenetic tree was constructed to study the evolutionary relationships using the neighbour-joining method.

Results: The variants of concern belonged to 26 species and there were 4 non-classified variants. All variants of concern showed 99.47–99.98% homology with the original Wuhan strain and demonstrated up to 60 mutations per variant. There were 1101 mutations identified among the variants of concern, with 458 synonymous and 583 nonsynonymous mutations. Specific mutations that were characteristically present in various SARS-CoV-2 lineages were identified, showing the microevolutionary genetic variations.

Conclusion: This study showed vast genetic variations of the Egyptian SARS-CoV-2 with characteristic mutations for the A, B and C lineages. The study contributes to a better understanding of the epidemiological and demographic variations of COVID-19 in Egypt based on SARS-CoV-2 genomic evolution and supports further investigation of SARS-CoV-2 contextbased vaccine development and preventive measures.

Keywords: SARS-CoV-2, COVID-19, genetic variation, variant, pathogenicity, transmissibility, BLASTN, BLASTP, Wuhan, Egypt

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Introduction

COVID-19 is a respiratory illness caused by SARS-CoV-2 and it may lead to respiratory distress and death (*1, 2*). SARS-CoV-2 is a novel virus related to a bat coronavirus that has never been previously isolated in humans. COVID-19 was first reported in Wuhan, China in December 2019, and 3 months later, WHO declared it a public health emergency of international concern because of its rapid global expansion and mortality *(1–3*).

SARS-CoV-2 is a single-strand, positive-sense ribonucleic acid (RNA) virus that belongs to the order Nidovirales of the Coronaviridae family. The ~30 kb genome of SARS-CoV-2 encodes 27 proteins, including 15 nonstructural, 4 major structural and 8 accessory proteins. The 3' terminus contains coding regions for 4 structural proteins [surface (S), membrane (M), nucleocapsid (N) and envelope (E)]. The 3' terminus encodes the longest open reading frame (ORF)1a/b, which encodes nonstructural proteins (*4, 5*).

Epidemiological data have shown that within a few months of the spread of COVID-19, many lineages of SARS-CoV-2 had emerged, which were characterized by various patterns of mutations. Some of these lineages were classified by WHO as variants of concern, such as Alpha, Delta and Omicron, based on characteristics such as enhanced rate of transmission, disease severity and neutralization of antibodies. Other lineages that spread less widely had mutations similar to variants of concern and may have clinical impact in the future; therefore, they were classified as variants of interest (e.g. Eta and Lambda) (*6*). Recently, the European Centre for Disease Prevention and Control nominated a new group of lineages, namely, variants under monitoring, which do not pose an immediate risk but may pose a threat to control of the pandemic in the future.

Many studies have shown specific mutations linked to geographical regions: Gly476Ser and Val483Ala are mainly observed in the United States of America (USA), whereas Val367Phe has been detected in China, France, Netherlands and Hong Kong Special Administrative Region (*4*). An early report stated that Egypt had the highest risk of SARS-CoV-2 among African countries, and on 14 February 2020, Egypt announced the first case

of COVID-19 in Africa (*1,7*). The pandemic in Egypt is heterogeneous in nature with mobile genetic variations among the variants of concern. Egypt, like other African countries, has a significant burden of non-COVID-19 disease which has led to reduced immunocompetence among a large portion of the population, and may have created a promising host environment for recombination in subsequently emerging viruses. Up until 24 March 2024, Egypt had reported 516 023 cases of COVID-19 with 24 830 deaths (*8*). However, previous reports suggest that COVID-19 in Egypt is under-reported, particularly cases that have been exported from Egypt to other countries. Therefore, the burden of SARS-CoV-2 in Egypt may be substantially greater than reported (*7*).

A high frequency of mutations in the SARS-CoV-2 genome was reported in Egypt in 2021 (*9*). ORF1ab and S and N were the most affected genes, and the most frequent mutation was D614G. Such mutations were detected during the first and second waves of COVID-19 in different countries (*9*). In 2022, it was found that all SARS-CoV-2 types isolated in Egypt were significantly mutated, with V5F, G823S, Q57H and D614G mutations (*1*). In this study, we conducted comprehensive mapping of the variations in the genomic characteristics of SARS-CoV-2 isolates from Egypt, which may contribute to a better understanding of the epidemiologic and demographic variations of COVID-19.

Methods

This was an *in silico* cross-sectional study that was part of a large project to determine the genetic evolution of SARS-CoV-2, based on time of isolation and geographic location. The first SARS-CoV-2 sequence (Wuhan strain, accession number NC_045512) is the reference sequence for studying the nucleotide changes and their corresponding amino acids.

The sequences of SARS-CoV-2 isolated in Egypt were retrieved from the National Centre for Biotechnology Information. The nucleotide changes were determined by comparing each sequence to the original Wuhan strain using nucleotide basic local alignment search tool (BLASTN) (https://blast.ncbi. nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_ TYPE=BlastSearch&LINK_LOC=blasthome).

The substituted nucleotides were subjected to further study to establish the effect of these changes on the corresponding proteins. The NCBI nucleotide website (https://www.ncbi.nlm.nih.gov/) was used to retrieve the proteins encoded by each SARS-CoV-2 genome. The effects of nucleotide substitutions on protein sequences were determined using protein basic local alignment search tool (BLASTP) (https://blast.ncbi. nlm.nih.gov/Blast.cgi?PROGR AM=blastp&PAGE_ TYPE=BlastSearch&LINK_LOC=blasthome), and all the mutations were reported. The effects of nucleotide changes were classified as follows: silent (synonymous) mutations, in which the changes did not lead to amino acid alterations; missense mutations, in which the

nucleotide changes led to changes in the amino acid sequences; insertion mutations, in which 1 or more nucleotides were added; and deletion mutations, in which there was a frameshift characterized by loss of 1 or more nucleotides.

A phylogenetic tree was constructed using the neighbour-joining method to study the evolutionary relationships among the SARS-CoV-2 isolates (*10*). The related taxa were clustered together in the bootstrap test (500 replicates) and reported as a percentage of replicate trees, shown next to the branches. The more similar variants were located near each other, while heterogeneous variants were at a distance (*11*). The maximum composite likelihood method was used to calculate the evolutionary distances (*12*) and correlated with the number of base pair substitutions per site. MEGA X software was used to conduct the evolutionary studies.

Simple statistical analysis was used to calculate the frequencies and percentages of each detected mutation. Ethical approval for the study was not needed.

Results

Two hundred SARS-CoV-2 variants of concern retrieved from NCBI on 11 January 2021 were aligned with the original Wuhan variant (NC_045512). The variants of concern belonged to 26 species and 4 were nonclassified variants (Figure 1). All variants of concern showed 99.47–99.98% homology with the NC_045512 variant, and there was an average of 60 mutations per variant.

There were 1101 mutations identified in the variants of concern, with 458 synonymous and 583 nonsynonymous mutations (Table 1). The most frequent mutations associated with most of the variants of concern were the synonymous point mutation C3037T, which was present in 88%, followed by C14408T in the ORF1a/b (87.5%) and C241T in the 5' untranslated region (UTR) (86%) (Figure 2). Five nonsense mutations were also noted in all the targeted variants of concern, which occurred in ORF7a, ORF7b and ORF8 (Table 1). Such mutation causes shortening of the encoded protein and may substantially affect its function. Six frameshift mutation were identified, including 4 deletions, 2 of them present in the S gene and 1 each in ORF7a and ORF8, and 2 insertions in ORF3a and ORF8 (Table 1). Several mutations appeared in noncoding regions between S gene and E gene, ORF6 and ORF7a, and ORF8 and ORF10 towards the 3' UTR (Table 1). The most common variants of concern in the sample population was AY.103 (23%), which was followed by AY.34 (21%). These were present during the third wave of SARS-CoV-2 (Figure 1).

The nonsynonymous point mutations with high frequency of detection in ORF1a/b included: C14408T, with substitution of proline with lysine at position 4715; A11201G, with substitution of threonine with alanine at position 3646; G15451A, with substitution of glycine with serine at position 5063; and C16466T, with substitution of proline with leucine at position

Figure 1. Variants enrolled in the study

Figure 1. Variants enrolled in the study

ORF = open reading frame; UTR = untranslated region

5401. These mutations occurred in > 75% of the aligned variants of concern (Figure 2).

As observed in the S gene, the most common nonsynonymous mutations included C21618G, with substitution of threonine with arginine at position 19, and T22917G, with substitution of lysine with arginine at position 452 (Figure 1). ORF3a had 1 common mutation C25469T (S26L), and several common mutations were found in the M gene, with the most common being G29402T (D377Y) (Figure 2).

The phylogenetic tree of the target sequences showed huge diversity of the variants of concern sequences with 64% relatedness of the variant with the accession number OK354415 to the new Omicron lineage. This variant belonged to the AY.43 lineage. B and C lineages were further from the Omicron variants. High similarity (97%) was shown in the clade between AY.11 (OL351405) and AY.83 (OL351406), followed by 93% for C17 (OK001885) and C36.3 (OK001885) (Figure 3).

Four unclassified variants of concern were also studied: OL351415 showed 39% similarity with AY.34; OK001871 and OK001874 were more related to B.1.1 and B.1.1.7; and OL351376 showed dissimilarity with all selected VOCs and Wuhan NC_045512 (Figure 3).

Several new mutations were assigned to A, B and C lineages. For example, in the A lineage, G4181T, C8986T, C10029T and A11332G with selective C884T, C1059T, C11109T, G15451A, G16381A, A16555T, A20268G, C21846T, A23116T, T26667C and C26936T mutations for the AY.43 lineage (Table 2). Some of the mutations observed in these earlier lineages were also present in the subsequent variants of concern with higher pathogenicity, such as Omicron.

Discussion

COVID-19 originated as a zoonotic disease (*13*) but has spread rapidly through human-to-human transmission and led to multiple waves of the disease worldwide (*14*). The unstable RNA genome of SARS-CoV-2 has resulted in a high frequency of mutations that have enhanced viral host entry and evasion of human immunity, enabling the virus to strike repeatedly with the emergence of novel variants (*17*). The origin of SARS-CoV-2 has not been definitively identified to date, but bats have been suggested as the primary source and pangolins as the intermediate host, based on the sequence homology of viruses isolated from these species that share identical genomic sequences with SARS-CoV-2 (*16*). SARS-COV-2 emerged as a highly infectious virus with an indicative basic reproduction number (R0) between 2 and 3 (*17*), thereby infecting large human populations in Europe, India and China, where mortality was higher than in the Middle East and Africa. Epidemiological data suggest that by December 2020, SARS-CoV-2 had invaded the entire world including Antarctica (*18*). It has been estimated that during the early symptomatic stage of COVID-19, viral load is highest in oropharyngeal secretions and patients can shed the virus even after symptoms have resolved, with a median duration of shedding of 20 days (*19*).

Studies have reported that SARS-CoV-2 has evolved via variations in the viral genome, with consequential effects on its transmission and pathogenicity (*20, 21*). Since the beginning of the pandemic, our group has comprehensively characterized such genomic variations in viruses isolated from countries such as China, India and Saudi Arabia (*3, 5*), including new variants such as Omicron (*22*). Genomic surveillance is an important step in identifying evolving viral lineages with potentially altered epidemiological characteristics, in order to mount an effective public health response (*23*). Since the beginning of the pandemic until January 2022,

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Figure 2. Common mutations associated with Egyptian SARS-CoV-2 variants of concern

Figure 3. Phylogenetic tree of selected Egyptian variants of concern compared with the original Wuhan variant and 2 Omicron variants

several variants of concern with varying pathogenicity were identified, including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529), which were responsible for multiple waves of COVID-19 (*24*). Such evolution has led to an estimated 50–80% increase in transmissibility, substantial reduction in neutralizing activity in natural and vaccine-induced immunity, and higher risk of reinfection (*25*). Therefore, early genomic surveillance and genomic variation studies have contributed to our understanding of the epidemiology and pathogenicity of SARS-CoV-2. There has been disparity in surveillance between high-income (78%) and low- and middle-income (42%) countries, which may have resulted from resource limitations and ineffective organization of public health systems (*23*). During the first wave of the pandemic, among the African nations, Egypt recorded the highest number of cases and mortality, after emergence of the first case on 15 February 2020 (*26*).

We studied genomic sequences of 200 variants of concern from Egypt up to 4 November 2021 that were available in the NCBI nucleotide database. After that date, COVID-19 was contained worldwide and began to decline, without any substantial wave except for that caused by the Omicron variant, which we retrieved later and used in the phylogenetic study. These 200 variants of concern demonstrated homology of 99.47–99.98% with the original Wuhan strain of SARS-CoV-2 with no single identical variants, suggesting that, during December 2019 to February 2020, the ancestral strain was completely replaced with new variants that had undergone gradual evolution. However, another study showed some similar sequences to the Wuhan strain (*27*). It was also realized that during the course of the pandemic the virus was continuously accumulating new mutations. We found 1101 mutations in 200 variants, which is higher than in earlier studies that reported mutation rates of 247/202 (*28*), 536/463 (*29*) and 116/95 (*27*). These differences are perhaps not unexpected as the earlier studies may have had different timelines. SARS-CoV-2 has evolved competitively over time, resulting in high mutation frequencies in the Delta and Omicron variants that came to dominate later severe waves of COVID-19 worldwide (*30*). Although most initial mutations were neutral and did not affect virulence or transmissibility of SARS-CoV-2, some mutations, such as those in S gene encoding the receptor binding domain, such as E484K, E484Q, K417T, K417N, N501Y, Y453F, iN439K, N440K, L452R and S477N, were key determinants of transmission and infectivity (*24*). In the 200 variants of concern in our study, although the number of mutations was high in ORF1ab ($n = 634$), because of its large size and important function of its encoded proteins, the number of synonymous and nonsynonymous mutations was nearly equivalent (304 and 330, respectively). One of the dominant, high-frequency nonsynonymous mutations in ORF1ab was C14408T, which altered the amino acid sequence

in RNA-dependent RNA polymerase, and potentially influenced the rate of viral replication and viral load in the host, as reported by Na et al. (*31*). However, in case of S gene, the number of nonsynonymous mutations (n = 105) was double that of synonymous mutations $(n = 59)$. Nonsynonymous mutations, such as L542R and D614G, which we reported as dominant in Egyptian SARS-CoV-2 isolates, can change the amino acid sequence and viral phenotype. This can confer an advantage to the virus in its interaction with the host and immune escape (*32*). Population-based phylogenetic studies with > 25 000 sequences have revealed that SARS-CoV-2 variants harbouring D614G mutation have substantially higher rates of transmission, infectivity and pathogenicity (*20*). D614G mutation is simultaneously associated with 3 other mutations: C241T, C3037T and C14408T (*33*). The spike protein makes an important contribution to the attachment, fusion and entry of SARS-CoV-2 into the host cell, and progressive mutation in this region indicates that the virus is evolving to be more competent for human-to-human transmission (*34*). With the heavily mutated spike protein of the Omicron variant (*32*), it is interesting to note that 24% of such nonsynonymous mutations were already present in the earlier lineages of SARS-CoV-2 that were identified in Egyptian isolates, as reported here.

We showed a gradual genomic evolution of different variants in the Egyptian population, where some common mutations were retained and critical new mutations were accumulated as an adaptive and competitive response towards dominance. This accords with the phylogenetic relationship in which 2 Omicron variants clustered together, covering all the different clades of previous lineages, therefore indicating a definitive microevolutionary genomic relationship. Some nonsynonymous mutations in M and N proteins have been suggested to induce suppression of interferon regulatory protein-3 and interfere with interferon production in hosts (*35*). The comparative data presented in our study are significant as they are indicative of all circulating SARS-CoV-2 variants in Egypt during the multiple waves of COVID-19, which varied demographically in time and space worldwide. The tracing and tracking of such SARS-CoV-2 variants and their gradual accumulation of critical mutations are likely to contribute to better understanding of the epidemiology of COVID-19 and its progression and severity in Egypt, along with the future course of viral evolution.

One limitation of our study was that some retrieved sequences were incomplete and had to be ignored to avoid misevaluation of the mutations.

Conclusion

We recommend in-depth study of the characteristic mutations associated with each variants of concern to assign new mutations to one of the known lineages, which could help with future laboratory typing instead of

in the Egyptian SARS-CoV-2 isolates, with characteristic mutations for the A, B and C lineages. The results may contribute to a better understanding of epidemiologic and demographic variations of COVID-19 in Egypt based

on genomic evolution of SARS-CoV-2.

highly expensive whole genomic sequencing. The genetic microevolutionary variations observed in this study support further investigation of context-based vaccine development and preventive measures for SARS-CoV-2 infection. This study has shown wide genetic variations

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Caractérisation génomique approfondie des variants du SARS-CoV-2 présentant des lignées hétérogènes en Égypte

Résumé

Contexte : La variation génétique du SARS-CoV-2 demeure un problème de santé publique dans le monde entier, car elle influe sur la pathogénicité et la transmissibilité du virus.

Objectif : Déterminer les mutations génomiques du SARS-CoV-2 isolées en Égypte.

Méthodes : Il s'agissait d'une étude transversale in silico de 200 variants préoccupants du SARS-CoV-2, ayant été extraits du National Centre for Biotechnology Information le 11 janvier 2021 et alignés sur la souche originale de Wuhan (NC_045512). L'outil de recherche d'alignement local de base nucléotidique a été utilisé afin de caractériser les variations nucléotidiques. Les protéines correspondantes ont été identifiées à l'aide de l'outil de recherche d'alignement local de base pour les protéines. Un arbre phylogénétique a été créé pour étudier les relations évolutives au moyen de la méthode Neighbour-joining.

Résultats : Les variants préoccupants appartenaient à 26 espèces et il y avait quatre variants non classés. Tous les variants préoccupants présentaient une homologie comprise entre 99,47 % et 99,98 % avec la souche d'origine de Wuhan et montraient jusqu'à 60 mutations par variant. Nous avons identifié 1101 mutations dans les variants préoccupants, dont 458 étaient synonymes et 583 non synonymes. Des mutations spécifiques qui étaient présentes de manière caractéristique dans diverses lignées du SARS-CoV-2 ont été détectées, montrant des variations génétiques microévolutives.

Conclusion : La présente étude a mis en évidence d'importantes variations génétiques du SARS-CoV-2 égyptien, avec des mutations caractéristiques des lignées A, B et C. Elle permet de mieux comprendre les variations épidémiologiques et démographiques de la COVID-19 en Égypte en fonction de l'évolution génomique du SARS-CoV-2. Cette étude appuie également la réalisation d'études plus approfondies sur la mise au point de vaccins et la mise en place de mesures préventives en fonction du contexte du SARS-CoV-2.

التوصيف اجلينومي املتعمق للنسخ املتحورة لفريوس كورونا-سارس2- يف مرص يكشف عن سالالت غري متجانسة

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اخلالصة

الحلفية: لا يزال التحور الجيني لفيروس كورونا–سارس–2 يشكل تحديًا للصحة العامة في جميع انحاء العالم، لانه يؤثر على قدرة الفيروس على اإلمراض ورسايته.

الأهداف: هدفت هذه الدراسة الى تحديد الطفرات الجينية في نسخ فبروس كورونا-سارس-2 المعزولة في مصر .

طرق البحث:كانت هذه دراسة مقطعية باستخدام املحاكاة احلاسوبية عن 200 نسخة متحورة مثرية للقلق من فريوس كورونا-سارس2- ر ، اسرُتجعت من المركز الوطني لمعلومات التكنولوجيا الحيوية في 11 يناير/ كانون الثاني 2021 ومطابقتها مع سلالة ووهان الأصلية (045512-NC). واستُخدمت أداة البحث الأساسية للمطابقة المحلية للنيوكليوتيد (BLASTN) لتحديد النَّسخ المُتحورة من النيوكليوتيد. وحُددت البروتينات المقابلة بأداة البحث الأساسية للمطابقة المحلية للبروتين (BLASTP). وقد أُعدت شجرة لتطور السلالات لدراسة العلاقات التطورية باستخدام طريقة ارتباط اجلار. **النتائج:** تنتمي نسخ الفريوس املتحورة املثرية للقلق إىل 26 نوًًعا، وهناك 4 نسخ متحورة غري مصنفة. وكانت مجيع النسخ املتحورة املثرية للقلق متاثلة بنسبة تتراوح بين 99.47٪ و99.98٪ مع سلالة ووهان الأصلية، وقد وصل عدد الطفرات في النسخة الواحدة إلى 60 طفرةً. وقد كشف التحليل عن 1101 طفرة يف النسخ املتحورة املثرية للقلق، منها 458 طفرة مرادفة و583 طفرة غري مرادفة. وقد رصد التحليل طفرات حمددة كانت موجودة بشكل مميز يف خمتلف سالالت فريوس كورونا-سارس،-2 وهو ما يظهر الطفرات اجلينية التطورية الدقيقة.

االستنتاجات:أظهرت هذه الدراسة اختالفات جينية واسعة النطاق لسالالت فريوس كورونا-سارس2- املرصية مع طفرات مميزة لسالالت A و B و C. وتسهم الدراسة يف حتسني فهم املختصني للتغريات الوبائية والسكانية لكوفيد19- يف مرص استناًًدا إىل التطور اجلينومي لفريوس كورونا- سارس،2- وتدعم مواصلة استقصاء تطوير اللقاحات وتدابري الوقاية القائمة عىل السياق لفريوس كورونا-سارس.2-

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